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(12) (19) (CA) **Demande-Application**

(21) (A1) **2,256,256**
(22) 1998/12/17
(43) 2000/06/17

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(51) Int.Cl.[6] A23K 1/16

(54) **COMPOSITION A LIBERATION CONTROLEE DE SUBSTANCES BIOACTIVES A USAGE ZOOTECHNIQUE**

(54) **COMPOSITION WITH CONTROLLED RELEASE OF BIOLOGICALLY ACTIVE SUBSTANCES FOR ZOOTECHNICAL USE**

(57) Compositions for zootechnical use for the administration of biologically active substances with nutritional and/or pharmacological properties, with delayed release, are described. The compositions comprise a vehicle constituted by a mixture of fats and waxes, wherein the biologically active substances are incorporated. The method for the production of the compositions comprises the melting of the mixture of fats and waxes, the incorporation of the biologically active substances in said molten mixture, and the subsequent solidification and fragmentation obtaining particles with dimensions of 400-500 m. The compositions of the invention are used for the activation of feeds, for the purpose of obtaining medicated and/or nutritionally integrated feeds, for instance with essential amino acids or vitamins.

ABSTRACT OF THE DISCLOSURE

Compositions for zootechnical use for the administration of biologically active substances with nutritional and/or pharmacological properties, with delayed release, are described. The compositions comprise a vehicle constituted by a mixture of fats and waxes, wherein the biologically active substances are incorporated. The method for the production of the compositions comprises the melting of the mixture of fats and waxes, the incorporation of the biologically active substances in said molten mixture, and the subsequent solidification and fragmentation obtaining particles with dimensions of 400-500 m. The compositions of the invention are used for the activation of feeds, for the purpose of obtaining medicated and/or nutritionally integrated feeds, for instance with essential amino acids or vitamins.

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COMPOSITION WITH CONTROLLED RELEASE OF BIOLOGICALLY ACTIVE SUBSTANCES FOR ZOOTECHNICAL USE.

BACKGROUND OF THE INVENTION

The present invention relates to a composition for zootechnical use for the oral administration with controlled release of biologically active substances having pharmacological and/or nutritional properties.

The present invention further relates to a method for the preparation of said composition, as well as the use of said composition as an additive to feeds, in order to obtain medicated and/or nutritionally integrated feeds.

Biologically active substances having nutritional properties (i.e. food integrators) and/or pharmacological properties are commonly administered to animals orally, generally added to the feeds.

In such administrations the biologically active substances undergo a chemical-enzymatic degradation during the transit time through the animal organism prior to reaching the intestine. Such degradation is quite severe in the case of ruminants, since transit time in the ruminant system is very high and due to the presence of the microbic flora.

The biologically active substances may also undergo a degradation during the feed enrichment phase and during the storage of the enriched feeds prior to their administration to the animals.

It is also important to mask the taste of the biologically active substances, particularly those with pharmacological properties, in order to make more attractive the medicated portions of feed to be administered to the animals.

Examples of substances commonly added to feeds are some vitamins. Vitamins are prone to being degraded prior to reaching the intestine of the animal. Such degradation is also caused by interference with the microflora which is present in the organs of the animals (and especially in the ruminal system of ruminants) which are traversed before the intestine is reached.

For instance, in the case of vitamin C, the rate of degradation during permanence in the rumen can even exceed 90%. More or less severe degradation is also experienced

with other vitamins, such as vitamin A, vitamin B6, vitamin B12, vitamin D and vitamin E. Particularly high is the degradation of the free form of vitamin E (DL-alpha-tocopherol); less high is the degradation of the esterified form (DL-alpha-tocopherylacetate).

Other examples of substances that are added to feeds and are subject to degradation are riboflavin, folic acid, niacin and choline. The latter plays an essential role in large lactiferous animals during the first phase of lactation, where a considerable mobilization of reserve fats takes place to compensate for energy shortages. The fatty acids accumulated in the liver are therein transformed into triglycerides, which in turn give rise to pathological hepatic steatosis. Choline is able to obviate such accumulation favoring the phospholipidic metabolism, and thus the lipoproteins that carry out the function of triglyceride transport and removal. The proper administration of choline improves the animal's hepatic functionality and productivity. However, unprotected choline is degraded into trimethylamine, an inactive substance that gives unpleasant flavor and odor to the milk. Such degradation can reach levels of about 70% of the quantity initially administered.

Other substances that are added to feeds and are subject to degradation prior to reaching the intestine of ruminants are amino acids. Enrichment with amino acids is important since milk production is particularly sensitive to the required quantity of plasmatic amino acids, essential for *de novo* synthesis of milk proteins at the mammary gland level. All amino acids necessary for the metabolism of milk proteins must be present in the blood in the quantity necessary for the proper "assembly" of the proteinaceous -cascous component.

Maximum proteinaceous efficiency is often compromised by the lack of two essential amino acids: L-lysine and DL-methionine. The massive administration of protein sources is not always able to obviate this problem. A protein excess can set off forms of competition upon absorption and/or tissue and cell utilization, for instance competition between L-triptophane and branched amino acids or between L-lysine and L-arginine. Moreover, the excess of nitrogenous substances, if not calibrated, entails an overload of ammonia defecation, aggravating environmental impact and hygienic conditions inside the stock farm, particularly if enclosed.

Furthermore, a shortage of amino acids, especially DL-methionine, limits the

growth, productivity and fertility of the animals.

Examples of pharmacologically active substances added to feeds and administered to animals are some antibiotics, both synthetic and fermentative; in many cases, the antibiotics so added, especially macrolidic or quinolinic ones, in addition to being degraded, also modify the taste of the feeds making them unattractive.

The need to improve the stability and preservability of feeds enriched with biologically active substances, for instance nutritional integrators, is all the greater, the higher the water content in the feeds. For example, liquid or semi-liquid mixtures with high water content, common in swine nutrition, are unstable because they are subject to very pronounced fermentative or hydrolytic transformations.

In order to reduce the aforesaid drawbacks, the proposal has been advanced of encapsulating some biologically active substances for zootechnical use with films of pH-sensitive materials, able to withstand the gastric environment, for instance polymers based on polyvinylpyrrholidone, vinyl polymers and copolymers, polyesters and polyamides, chemically modified cellulose, polypeptidic agents and starches, thereby obtaining a certain protection of the active substances and their delayed release in the intestine of the animals.

Such solutions present some drawbacks in that the particles of biologically active substances so encapsulated occasionally have significant dimensions, and as such are subjected to the animal's mastication and, in the case of ruminants, to prolonged periods of permanence in the rumen. Moreover, the use of polymers as film producing-protective agents has high costs and, in the case of synthetic polymers, introduces non physiological substances in the animal diet.

It has also been proposed to protect some biologically active substances for zootechnical use, allowing their delayed release in the intestines of animals, incorporating the substances in oils (for instance hydrogenated soy or cottonseed oil, or coconut oil), fatty acids or triglycerides. However, solutions of this kind do not guarantee a sufficient resistance of the biologically active substances to the chemical-enzymatic attack whereto they are subjected in the animal organism prior to reaching the intestine, which attack is particularly prolonged in the case of ruminants.

The applicant has now found that the incorporation of biologically active substances, having pharmacological and/or nutritional properties, in mixtures

comprising fatty acids or esters of fatty acids in combination with waxes allows a high resistance of the biologically active substances in the gastric system of the animals, as well as a controlled, delayed and calibrated release of the substances themselves. Such release, for instance in the case of ruminants, takes place mostly in the post-ruminal area, from the abomasal area to the small intestine, wherein the absorption of the non degraded administered substance thus takes place.

SUMMARY OF THE INVENTION

A subject of the present invention is therefore a composition for zootechnical use for the oral administration with controlled release of one or more biologically active substances having pharmacological and/or nutritional properties. In particular, the composition of the present invention is in the form of particles, preferably with dimensions from 400 to 2500 m, more preferably from 500 to 1400 m, which comprise a vehicle wherein said one or more biologically active substances are incorporated. The vehicle preferably has a melting temperature of at least 40°C and it comprises:

- a component (A) constituted by one or more fatty acids and one or more esters of a fatty acid, and
- a component (B) constituted by one or more waxes.

The component (A) is present in the composition in an amount equal to 10-90% of weight, preferably 30-80% of weight, more preferably 35-75% of weight, with respect to the total weight of the vehicle. The component (B) is present in the composition in an amount equal to 10-90% in terms of weight, preferably 20-70% of weight, more preferably 25-65% of weight, with respect to the total weight of the vehicle.

Some examples of components (A) are the following:

- hydrogenated oils preferably saturated, with melting temperature from 50 to 85°C and saponification number from 120 to 205;
- fatty acids, natural or hydrogenated or partially hydrogenated, having melting temperatures from 57 to 70°C and saponification number from 150 to 230;
- esters of fatty acids, of vegetable or animal origin, for instance mono-, di- and triglycerides, with melting temperature from 45 to 70°C and saponification number

from 175 to 205.

Preferably the component (A) of the vehicle comprises glycerides or long chained fatty acids (for instance C12-C22), which can be of vegetable origin (for example hydrogenated palm, soy, cottonseed, various seeds, olive, castor and sesame) or of animal origin. Hydrogenated oils of vegetable origin are preferred.

In U.S. Pharmacopoeia, hydrogenated oils of vegetable origin are classified according to their physical properties, regardless of their origin. Type I fats are better indicated for application in the present invention, being characterized by a melting point ranging from 57 to 70°C, iodine value lower than 5 and saponification number from 175 to 205 according to the ASTM D1387 standard.

In the composition of the present invention the component (B) is constituted by one or more natural or synthetic waxes. Natural waxes can be animal, vegetable or mineral. It is preferable to use natural waxes with melting point from 50 to 86°C.

Particular examples of usable waxes according to the present invention are: carnauba wax, beeswax, esparto wax, ceresine, ozocerite (for example, not refined), paraffin waxes or micro-crystalline waxes.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

In a preferred composition the component (B) of the vehicle is constituted by or comprises a micro-crystalline wax. Particularly preferred is a composition wherein the component (B) is constituted for at least 50% of its weight by one or more non-saponifiable micro-crystalline waxes with melting temperatures from 54 to 102°C, preferably from 90 to 100°C, and carnauba wax.

The term wax chemically means all substances essentially constituted by saturated fatty acids, generally ranging from C12 to C30, by fatty alcohols, generally ranging from C12 to C30, by esters between the aforementioned fatty alcohols and the fatty acids, by a minority component of triglycerides, and by hydrocarbons. The latter are present in variable proportions in natural, animal and vegetable waxes, with length ranging from C19 to C31, whilst they constitute the dominant fraction of mineral waxes.

Beeswax is formed by a mixture of linear monohydric alcohols with even Carbon number, from C26 to C36, such as cerilic alcohol (C26) and miricilic alcohol (C36),

esterified by linear fatty acids with even Carbon number up to C36, with presence of ricinoleic acid (C18 hydroxyacid). Examples of such esters are traicontanol hexadecanoate and hexacosanol hexacosanoate. Such esters are mixed with about 20% by weight of linear hydrocarbons (paraffins) with odd Carbon number, from C21 to C33. Also present are about 6% of unidentified substances, in addition to propolis and pigments. U.S. Pharmacopoeia describes yellow wax, obtained through primary refinement, and wherefrom white wax, useful in cosmetic applications, is obtained through bleaching with peroxides. The melting point of beeswax, regardless of its degree, varies from 62 to 65°C, the saponification number from 87 to 104 in accordance with ASTM D1387.

Carnauba wax comes from the exudate of the leaves of the palm by the same name, classified as Copernicia Prunifera (Muell). It contains waxy esters, i.e. esters of saturated fatty acids with saturated or hydroxylated fatty alcohols with average length of 12 Carbon atoms. The melting point of carnauba wax varies from 82 to 85.5°C, the saponification number from 78 to 89 in accordance with ASTM D1387.

Esparto wax, chemically constituted mostly by hydrocarbons (entriacontane C31) and by the classic waxy esters, is obtained as a by-product in paper processing. Candelilla wax is obtained from the green parts of many Euphorbiaceae and it is mostly constituted by hydrocarbons (47-57%), so it represents the natural component most closely resembling mineral paraffin waxes.

Among waxes constituted mostly or completely by hydrocarbons, ozocerite or ceresine are noted, which correspond to increasing levels of refinement of the same material.

Micro-crystalline waxes, paraffin waxes and carnauba wax are described in U.S. Pharmacopoeia as excipients and as such allowed for human pharmaceutical use. They can be obtained by successive purification from crude oil or from its heavy fractions, or by pyrolysis of lignites or also by Fischer-Tropsch synthesis from CO and H. They are classified according to their chemical-physical characteristics, with increasing melting point and hardness. To obtain products with high melting points, various physical processes are used: differential melting, fractionated crystallization, filtration with solvent mixtures, etc. In the composition of the present invention the component (B) is present in amounts ranging from 10 to 90% of the weight of the vehicle. High

percentages of the component (B) allow greater protection of the biologically active substances contained in the composition. In the case of low percentages of the component (B), it is advisable for the weight ratio between micro-crystalline wax and the other natural waxes to be no lower than 2:1.

The biologically active substances that, according to the present invention, can be incorporated in the vehicle comprising the components (A) and (B), can be substances with pharmacological and/or nutritional properties, such as:

- essential amino acids, for instance DL-methionine and L-lysine, or choline, or their salts;
- vitamins, for instance vitamins A, B1, B2, B6, B12, C, E and PP, or similar precursors of metabolic intermediates;
- probiotic substances, for instance Lactobacilli and Bifidobacteria;
- antibiotic substances (for instance of the amphenicolic, tetraciclinic, quinolonic, fluoroquinolonic, macrolidic or sulphonamide type), antihelmintic, antiprotozoic, antidiarrhoic or antimycotic substances.

The biologically active substances that are incorporated in the vehicle comprising the components (A) and (B) preferably have dimensions of 40-100 m, more preferably of 50-70 m. Particularly advantageous results are reached by obtaining the desired dimensions by micronisation.

The one or more biologically active substances can be present in the composition of the present invention in a quantity that varies from 0.01 to 60% by weight with respect to the weight of the vehicle. For example the quantity can be from 2 to 35% by weight with respect to the weight of the vehicle, or from 5 to 20% by weight with respect to the weight of the vehicle.

In the case of essential amino acids, such as DL-methionine or L-lysine, or in the case of choline or of their salts, the quantity is preferably from 20 to 50%, more preferably from 25 to 45% by weight with respect to the weight of the vehicle.

In the case of vitamins, biotin, pantothenic acid, nicotinic acid or nicotinamide, the quantity is preferably from 0.01 to 35%, more preferably from 1 to 25% by weight with respect to the weight of the vehicle.

In the case of probiotic substances, such as Lactobacilli or Bifidobacteria, the quantity is preferably from 2 to 30%, more preferably from 5 to 20% by weight with

respect to the weight of the vehicle.

In addition, the composition of the present invention can be modified with conventional additives, preservatives and anti-oxidizers that are compatible with animal administration and are used in animal husbandry to increase the stability of feed formulations. Typical preservatives include: thymerosalt, chlorbutanol, methyl, ethyl, propyl or butyl parabene. Typical anti-oxidizers of the oily phase include: alpha-tocopherol, alpha-tocopheryl acetate, B.H.T. and B.H.A.

The compositions according to the present invention can be prepared with a process that constitutes a further subject of the present invention and comprises the following operations:

(a) preparing a vehicle by melting a mixture comprising the component (A) and the component (B),

(b) incorporating in the molten vehicle thus obtained one or more biologically active substances, preferably with the aforementioned dimensions,

(c) subjecting to solidification and fragmentation the molten vehicle incorporating one or more biologically active substances,

(d) subjecting the particles thus obtained to sifting.

Phase (c) can be effected for instance by means of spray-cooling or by means of atomization; in both cases, cooling can be obtained, for instance, with nitrogen counter flow. Particularly advantageous is ultrasound atomization.

With the aforesaid process, particles are obtained of preferably spheroidal shape and preferably with dimensions of 400-2500 m, more preferably 500-1400 m.

The compositions of the present invention are employed in the field of animal husbandry, since they can be used by adding them to feeds to obtain medicated and/or nutritionally integrated feeds.

The feeds thus prepared can be administered with particularly advantageous results to ruminants since the biologically active substances contained therein are released in a controlled fashion (delayed and calibrated) in the post-ruminal system of the animals. The quantity of biologically active substances administered to the animal essentially reaches the post-ruminal system with no degradation and therefore it can be absorbed and used effectively.

Moreover, the compositions of the present invention can be advantageously

employed for the effective enrichment of feeds diluted in water (swills), as well as to improve the palatability of the medicated rations.

The examples that follow serve further to illustrate the invention and are not to be considered absolutely limiting.

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EXAMPLE 1 (comparative)

SATURATED FATTY ACID AND CRYSTALLINE METHIONINE

120 g of stearic acid, melting point 69.6°C, iodine No. = 0, acidity No. = 197.2 are melted at the temperature of 85°C and mixed with 80 g of crystalline DL-methionine, maintained under agitation for 30 minutes until the mixture is homogenized. Spraying is then started, introducing a nitrogen counter flow for cooling. The product is collected on a vibrating sieve composed of an upper net of 2200 m and a lower net of 600 m.

The particles obtained have the appearance of spheroidal micro-granules, with grain size ranging from 1400 m (max top 1%) and 500 m (max top 5%)

EXAMPLE 2 (comparative)

SATURATED FATTY ACID AND MICRONESIA METHIONINE

Example 1 is repeated with the same reactants and operating procedures, with the exception that the DL-methionine is previously Micronesia to a grain size whereby 100% pass through the 63 m sieve, obtaining particles sized 1400-500 m.

EXAMPLE 3 (comparative)

SATURATED TRIGLYCERIDE AND MICRONESIA METHIONINE

120 g of hydrogenated palm oil, melting point 54-57°C, iodine No. = 1 max, acidity no. = 8 max, average fatty acid composition of 3% ministic acid (C14:0), 26-30% of palmitic acid (C16;0) and 58-68% of stearic acid (C18;0) are melted at the temperature of 85°C and mixed with 80g of Micronesia DL-methionine, maintained under agitation for 30 minutes until mixing is complete. Spraying is effected in nitrogen counter flow and the product is collected on a vibrating sieve composed of two nets of 2200 and 600 m. The particles appear as spheroidal micro-granules of grain size ranging from 1400 m (max top 1%) and 500 m (max top 5%).

EXAMPLE 4

SATURATED TRIGLYCERIDE WITH NATURAL WAXES AND MICRONESIA METHIONINE

Example 3 is repeated under the same conditions, with the difference that the quantity of hydrogenated palm oil is 108 g and that said oil is mixed while heated with 12 g of carnauba wax, obtaining, after treatments identical to those of Example 3, particles sized 1400-500 m.

EXAMPLE 5

MICRO-CRYSTALLINE WAX WITH SATURATED TRIGLYCERIDE AND MICRONESIA METHIONINE

100 g of micro-crystalline wax, melting point 84-87°C, mixed with 20g of the same hydrogenated palm oil used in Example 3, are treated according to the procedures already set out in the previous examples (from 2 to 4), obtaining particles containing 40% of DL-methionine by weight.

EXAMPLE 6

DETERMINING METHIONINE TITER

The methionine content of Examples 1 through 5, and similarly for the subsequent Examples, is determined by titration in non aqueous environment after dissolving the particle in non aqueous acid environment and at the temperature of 85°C.

The dissolution of the sample (containing about 140 mg of methionine) is effected with 50 ml of glacial acetic acid, whereto are added about 3 ml of formic acid, heating to 85°C. Then titration is performed with 0.1 N perchloric acid with potentiometer, using the following computational formula:

$$\text{Methionine (\%)} = 100 \times (149.2 \times 0.1 \times A) / Pc$$

where

149.2 = molecular weight of methionine

0.1 = normality of perchloric acid

A = ml of 0.1 N perchloric acid consumed

Pc = weight of the sample in mg

For examples 1-5, values around 40% are obtained. The method is also applied to the examples that follow to measure the quantity of residual DL-methionine in resistance tests *in vitro* and *in vivo*.

EXAMPLE 7

RESISTANCE TESTS - *IN VITRO* METHOD

The degree of protection obtained incorporating the biologically active principle in the component (A) (fat) and/or in the component (B) (wax) is determined by measuring the decrease in the concentration of the active ingredient (methionine) after 8 hours of permanence in a buffer solution with pH 6.8 at the temperature of 39°C. In a glass bottle, 2 g of particles obtained incorporating the active ingredient in the

component (A) and/or in the component (B) are added. During the 8 hours of the test, taken as arbitrary reference to simulate an average permanence in the rumen, moderate agitation is applied 2-3 times, maintaining the indicated temperature with double boiler. At the end the solution is filtered on nylon filter, then bottle and filter are washed with 250 ml of buffer solution. Lastly, the quantity of active ingredient is determined, by means of potentiometric titration as described in Example 6.

The degree of protection expresses the ratio between the titer of the sample at the end of the test, washed and dried in a stove, with respect to the initial titer at time 0:

Degree of protection (%) = 100 x Tb / Ta

where

Tb = active ingredient (methionine) titer after 8 hours in pH 6.8 solution at 39°C

Ta = initial active ingredient (methionine) titer.

The particles of the Examples from 1 to 5 were considered, obtaining a good correlation with the data obtained from *in vivo* tests. Thus it is evident that an excellent simulation of gastric protection can be obtained by means of non invasive tests, easily accessed and inexpensive.

EXAMPLE 8

RESISTANCE TESTS - *IN VIVO* METHOD

Testing is conducted in accordance with the guidelines set out in the publication by various authors in Zoot. Nutr. Anim. (1994) 20:281-91. The test consists of the nylon bag technique which entails the abomasal insertion of the sample to be tested. The details are described in the guidelines provided by the Proteins in Polygastric Feeding commission of the ASPA (1994) which follows the French PDI system (NRA, 1988), partly modified based on appropriate indications of the literature (Susmel and Stefanon, 1987; Susmel et al., 1993b).

For the test, 3 Italian Frisian bovines were used, provided with ruminal fistula in dry physiological state.

Incubation times were 0, 2, 4, 6, 8, 12, 16, 20, 36, 48 and 96 hours. The dry content (Table 1) is measured by weighing the bag previously dried in stove according to standardized procedure, whilst the methionine content (Table 2) was determined according to the method described in Example 6.

The results are shown in Tables 1 and 2.

Example 1 Example 2 Example 3 Example 4 Example 5

Table 1 - total dry substance content (fat and/or wax + DL-methionine)

	Example 1	Example 2	Example 3	Example 4	Example 5
a (%)	9,7	4,2	6,3	5,1	6,0
b (%)	31,5	29,3	41,3	51,2	29,85
a+b (%)	41,2	33,5	47,6	56,3	35,9
c (%)	26,25	7,4	2,5	1,9	2,2
DG (6%h)	35,1^	20,1 ^{B}	19,0 ^{C}	16,9 ^{D}	13,55 ^{E}
DG (8%h)	33,6^	18,0 ^{B}	15,8 ^{BC}	14,5 ^{CD}	12,1 ^{E}

Table 2 - DL-methionine content

	Example 1	Example 2	Example 3	Example 4	Example 5
a (%)	19,3^	11,5 ^{B}	14,2^ ^{B}	9,4 ^{E}	9,5 ^{B}
b(%)	70,1 ^{B}	65,1 ^{B}	64,7 ^{B}	69,7 ^{B}	49,5 ^{C}
a+b (%)	89,4^	76,6 ^{B}	78,9 ^{B}	79,1 ^{B}	59,0 ^{C}
c (%)	29,6^	8,2 ^{E}	4,9 ^{C}	4,2 ^{C}	3,7 ^{C}
DG (6%h)	77,2^	48,8 ^{B}	43,1 ^{C}	37,9 ^{D}	28,3 ^{E}
DG (8%h)	74,1^	44,2 ^{B}	38,6 ^{C}	33,2 ^{D}	25,1 ^{E}

a (%) = immediately degradable fraction

b (%) = slowly degradable fraction

a+b (%) = total degradable fraction

c (%/h) = hourly degradation rate

DG (6%/h) = effective degradability with kp = 6%/h

DG (8%/h) = effective degradability with kp = 8%/h

Statistical analysis was performed by means of GLM and the differences were compared by means of Schoffe's test (SAS, 1994).

A = significance 0

B = significance 1

C = significance 2

D = significance 3

E = significance 4

F = significance 5

The immediately degradable fraction, a (%) corresponding to time 0, is obtained by washing the sample in cold water for 15 minutes, followed by centrifuging.

The slowly degradable fraction, b (%) is obtained from the difference between the final asymptotic weight (placed at 72 hours) and the immediately degradable fraction a (%). The value expressed as c (%/h) in practice corresponds to a percent degradation rate as hourly average.

The kinetic degradation parameters are defined with a, b and c, whilst the constant k = 0.06 or k = 0.08 corresponds to high speeds of ruminal transits equal to 6%/h or 8%/h, typical of concentrates. In other words, kp = 6% refers to an average transit of 16-28 hours, whereas kp = 8%/h refers to a ruminal transit corresponding to 13-14 hours.

The value of degraded portion (DG) is obtained using the formula:

$$DG = a + (b \times c) / (c + k)$$

To high DG values corresponds a low ruminal degradation protection. The quantity bypassing the rumen can be assumed equal to 100 - DG. Analyzing the values obtained in the tests, valid indications are obtained on the protective capacity of the substances used to incorporate the biologically active substance.

The data in Table 1 refer to the total quantity of particles (vehicle + active ingredient) not degraded during permanence in the rumen. They show poor resistance of the fatty acid (examples 1 and 2) and of the saturated glyceride (example 3) not used in combination with waxes. Greater resistance is observed with saturated triglyceride employed in combination with carnauba wax (example 4). Even greater resistance is observed with the saturated triglyceride used in combination with micro-crystalline wax (example 5).

EXAMPLE 9

PREPARATION BY MEANS OF ATOMIZATION

236 kg of hydrogenated palm oil, melting point 54-57°C, iodine No. = 1 max, acidity No. = 8 max, with average fatty acid composition of 3% miristic acid (C14;0), 26-30% palmitic acid (C16;0) and 58-68% stearic acid (C18;0), are loaded in reactor with 97.4 kg of carnauba wax and with 240 kg of Sasolwaks C 80 micro-crystalline mineral wax, then heated with steam jacket to the temperature of 85°C and mixed in the molten state.

These components, maintained under agitation at the temperature of 85°C, are enriched with 1.6 kg BHT, and then with 425 kg of Micronesia DL-methionine with grain size 63 m for 30 minutes.

The mixture obtained is then introduced in an atomizer, fed with a constant flow of 15 kg/min, with nitrogen counter flow equal to 0.1 - 0.2 l/kg at the external temperature of 5-12°C and equal to 0.5-0.7 l/kg at the external temperature of 20-30°C.

The particles are lastly collected for the analysis of the composition, which shows a concentration of DL-methionine equal to about 40% of weight. Average particle dimensions are retained in the range between 2200 and 600 m by means of sifting on sieves having the dimensions indicated above.

EXAMPLE 10

PREPARATION BY MEANS OF SONICATION (ULTRASOUNDS)

A heat-mixed mixture containing the quantity of ingredients in Example 9 and prepared with identical procedure constitutes the constant supply for an ultrasound atomizer (Tecnea, Castelguelfo - Bologna) adjusted to the intensity of 50 kHz, power 1800 Watts.

Composition is similar to that of Example 9 and average particle size is retained in the range from 2200 to 600 m by means of sifting on sieves of the dimensions indicated above.

EXAMPLE 11

COMPOSITION CONTAINING VITAMIN PP

100 kg of hydrogenated palm oil and 250 kg of stearic acid, with the characteristics described in Example 9, are loaded in reactor with 139 kg of carnauba wax and with 200 kg of Sasolwaks C 80 micro-crystalline wax, and heated with steam jacket to the temperature of 85°C and then mixed. The mixture thus obtained in the

molten state is enriched with 1.0 kg of BHT and 2.0 kg of Kavarom L. (H. & R. - Haarman and Reimer - Germany), and then with 408 kg of Micronesia vitamin PP, with grain size 63 m for 30 minutes.

The particles are then obtained with similar procedure to that of Example 9, thus with similar morphology.

EXAMPLE

COMPOSITION CONTAINING ERYTHROMYCIN THIOCYANATE

519 kg of monoglyceride GMS (Faci S.p.A. - Carasco - Genoa) and 380 kg of carnauba wax are loaded in reactor and heated with steam jacket to the temperature of 85°C and then mixed. The mixture thus obtained in the molten state is enriched with 1.0 kg of BHT and then with 100 kg activity of erythromycin thiocyanate, and mixed for 30 minutes.

Particles are lastly produced with a procedure similar to that of Example 9.

EXAMPLE 13

COMPOSITION CONTAINING PROBIOTICS

400 kg of oil of stearic acid, 250 kg of carnauba wax and 240 kg of type II paraffin wax are loaded in reactor, then heated with steam jacket to the temperature of 65°C and mixed in the molten state.

These components, maintained under agitation at the temperature of 85°C, are enriched with 1.0 kg of BHT, mixed for 30 minutes, and then enriched in the molten state with 100 kg of Lactobacillus acidophilus and mixed for 30 minutes.

Particles are lastly produced with a procedure similar to that of Example 9.

WHAT IS CLAIMED

1. Composition for zootechnical use for the oral administration with controlled release of one or more biologically active substances, having pharmacological and/or nutritional properties, said composition being in the form of particles comprising a vehicle in which said one or more biologically active substances are incorporated, said vehicle comprising:
- a component (A) constituted by one or more fatty acids and one or more esters of a fatty acid and,
- a component (B) constituted by one or more waxes,
each of the components (A) and (B) being present in quantities equal to 10-90% by weight with respect to the total weight of the vehicle.

2. Composition according to claim 1, in the form of particles with dimensions from 400 to 2500 m.

3. Composition according to claim 1 and/or 2, wherein the vehicle has a melting temperature of at least 40°C.

4. Composition according to one or more of the previous claims, wherein the vehicle is constituted for 30-80% of its weight by the component (A) and for 20-70% by the component (B).

5. Composition according to one or more of the previous claims, wherein the component (A) is a prevalently saturated hydrogenated oil, having melting temperature from 50 to 85°C and saponification number form 120 to 205.

6. Composition according to one or more of the claims from 1 to 4, wherein the component (A) is a fatty acid having melting temperature from 57 to 70°C and saponification number from 150 to 230.

7. Composition according to one or more of the claims from 1 to 4, wherein the component (A) is an ester of a fatty acid, said ester having a melting temperature from 45 to 70°C and saponification number from 175 to 205.

8. Composition according to claim 7, wherein the component (A) is a mono-, di- or triglyceride.

9. Composition according to one or more of the previous claims, wherein the component (B) is a natural wax having melting temperature from 50 to 86°C.

10. Composition according to one or more of the claims from 1 to 8, wherein the component (B) is a wax chosen from among carnauba wax, beeswax, esparto wax, ceresine, ozocerite, paraffin wax and micro-crystalline wax.

11. Composition according to claim 10, wherein the component (B) is constituted for at least 50% of its weight by one or more micro-crystalline waxes.

12. Composition according to one or more of the previous claims, wherein the one or more biologically active substances are chosen from among DL-methionine, L-lysine, choline and their salts.

13. Composition according to one or more of the claims from 1 to 11, wherein the one or more biologically active substances are vitamins or probiotic substances.

14. Composition according to one or more of the claims from 1 to 11, wherein the one or more biologically active substances are antibiotic, antihelmintic, antiprotozoic, antidiarrhoic or antimycotic substances.

15. Method for the preparation of a composition according to one or more of the previous claims, comprising the following operations:

(a) preparing a vehicle by subjecting to melting a mixture comprising a component (A) constituted by one or more fatty acids and one or more esters of a fatty acid, and a component (B) constituted by one or more waxes,

(b) incorporating in the molten vehicle thus obtained one or more biologically active substances,

(c) subjecting to solidification and fragmentation the molten vehicle incorporating one or more biologically active substances and,

(d) subjecting to sifting the particles thus obtained.

16. Method according to claim 15, wherein phase (c) is effected by means of spray-cooling or by means of atomization.

17. Method according to claim 16, wherein phase (c) is effected by means of ultrasound atomization.

18. Use of a composition according to one or more of the claims from 1 to 14 for addition to feeds for the purpose of obtaining medicated and/or nutritionally integrated feeds.